

Bacterial processing of glucose modulates *C. elegans* lifespan and healthspan

Supplementary Figures and Tables

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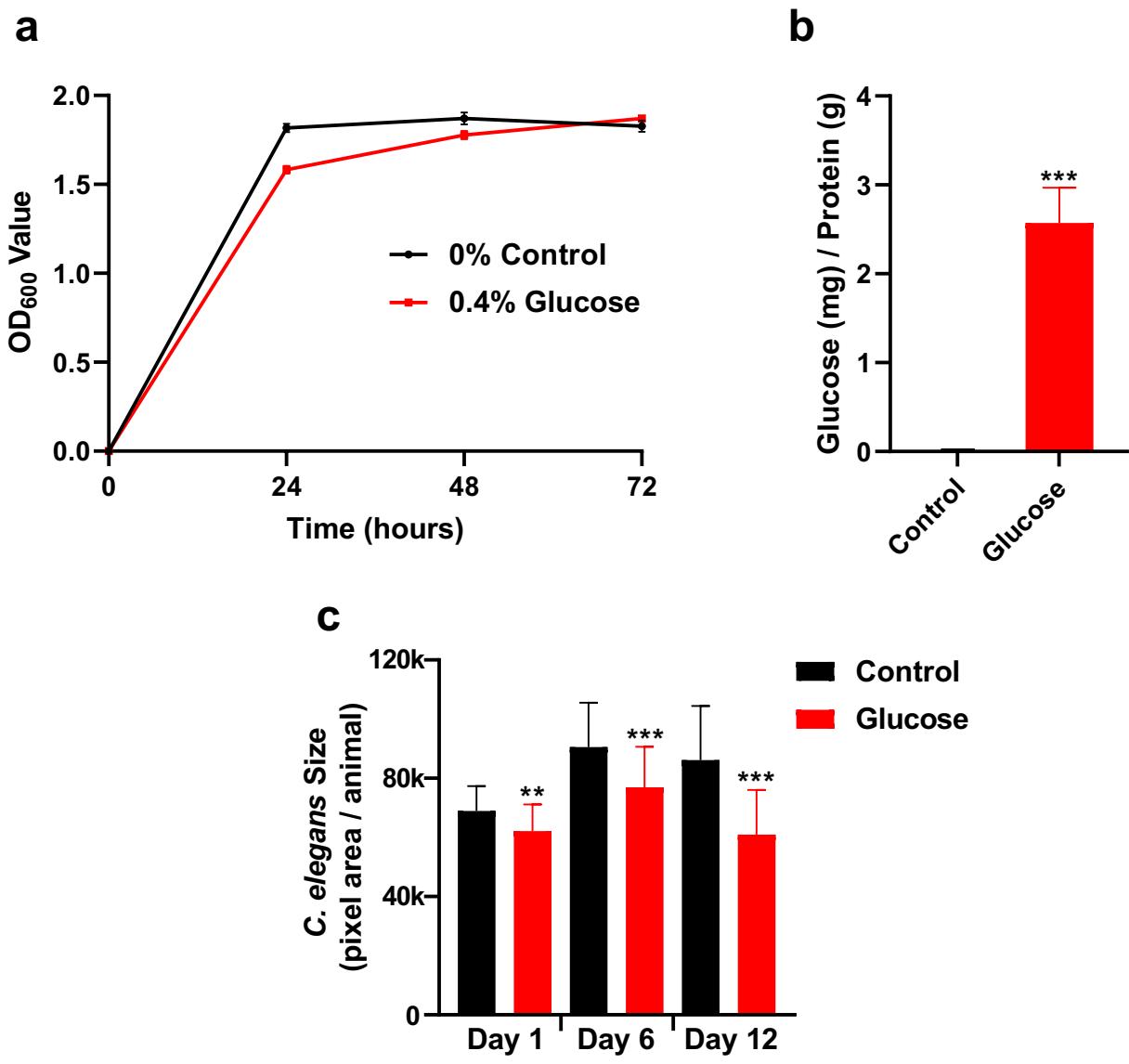
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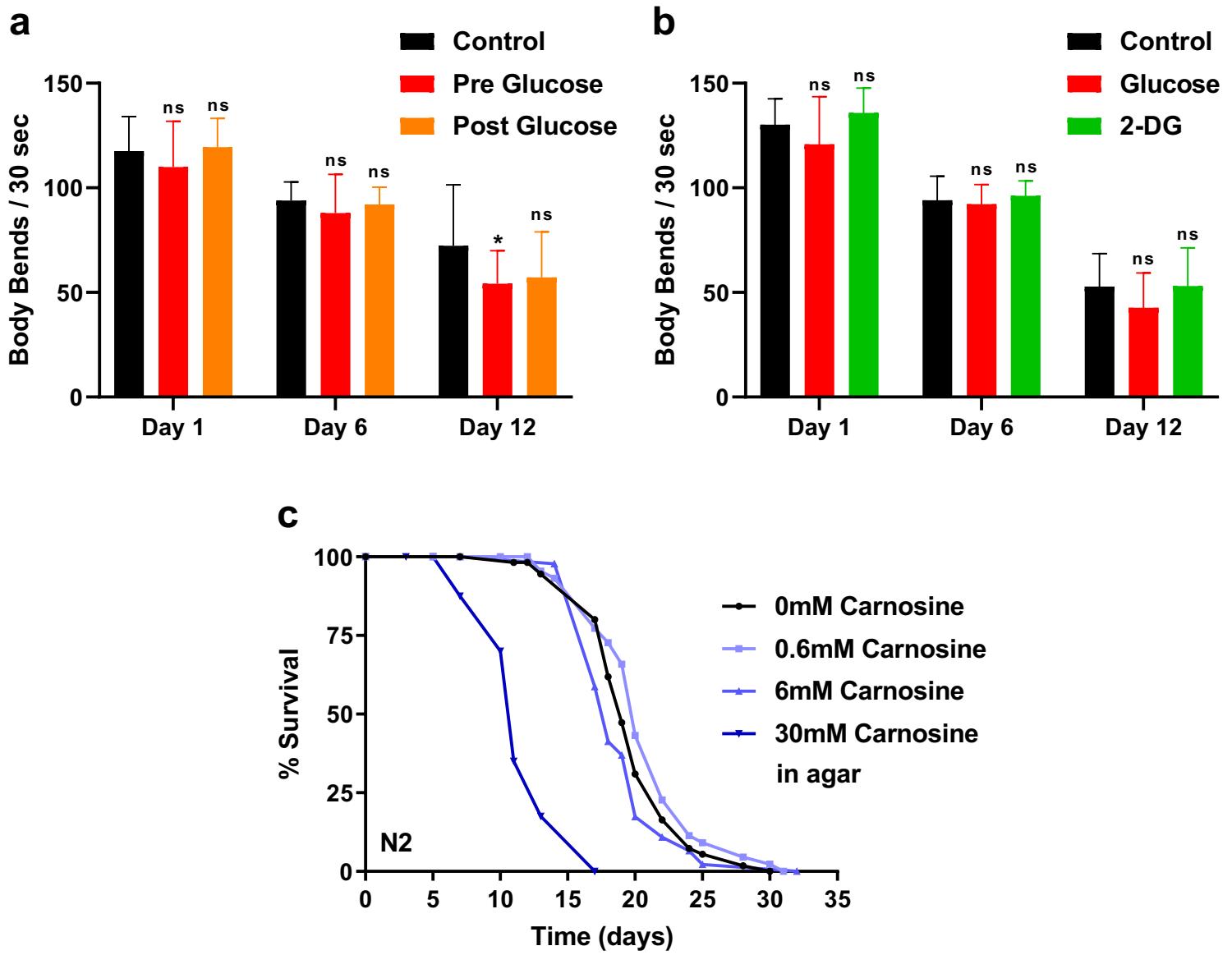
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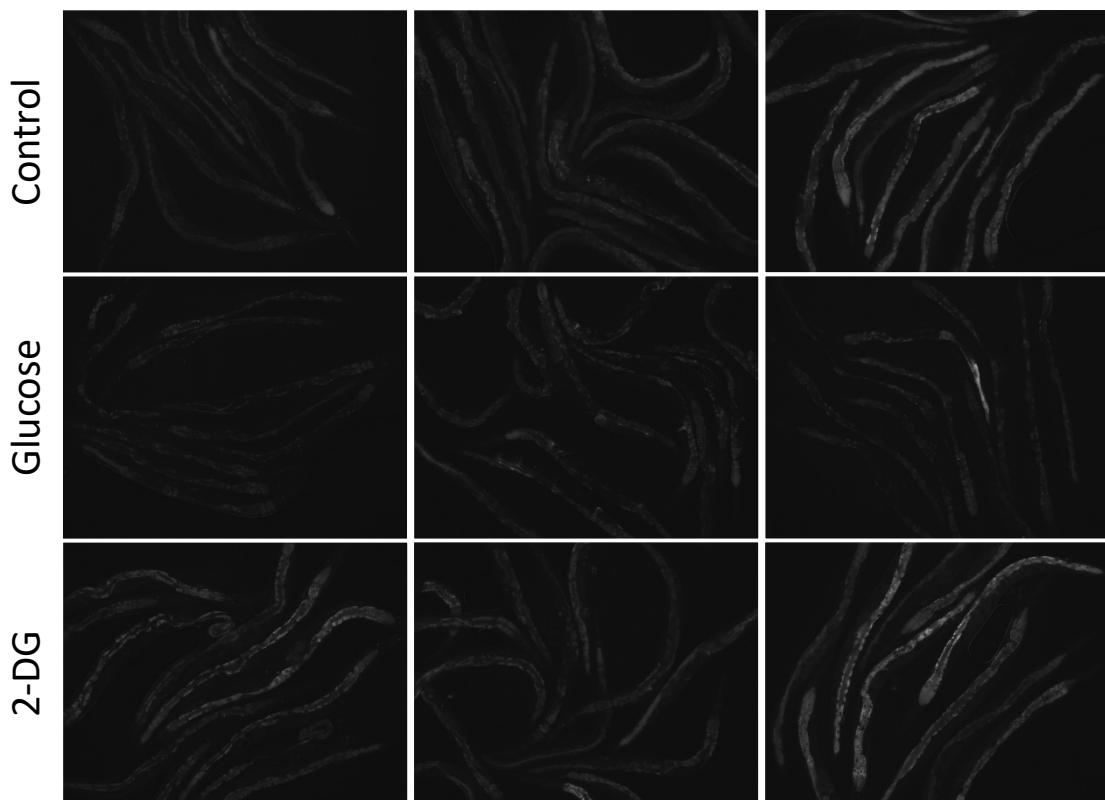
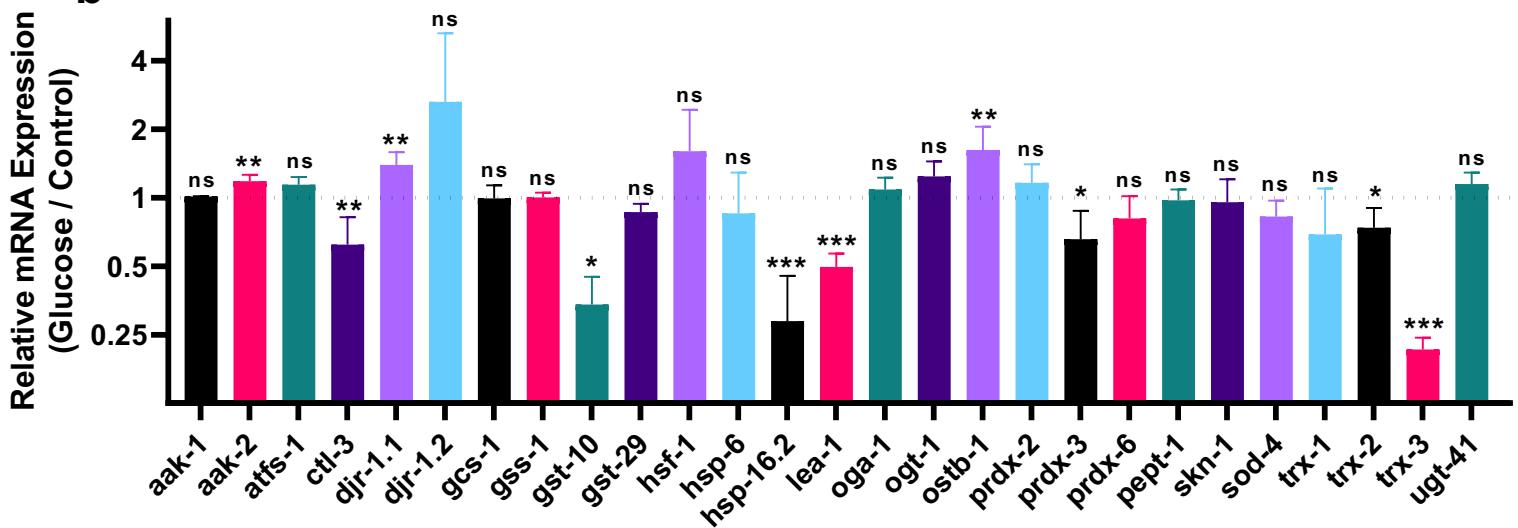
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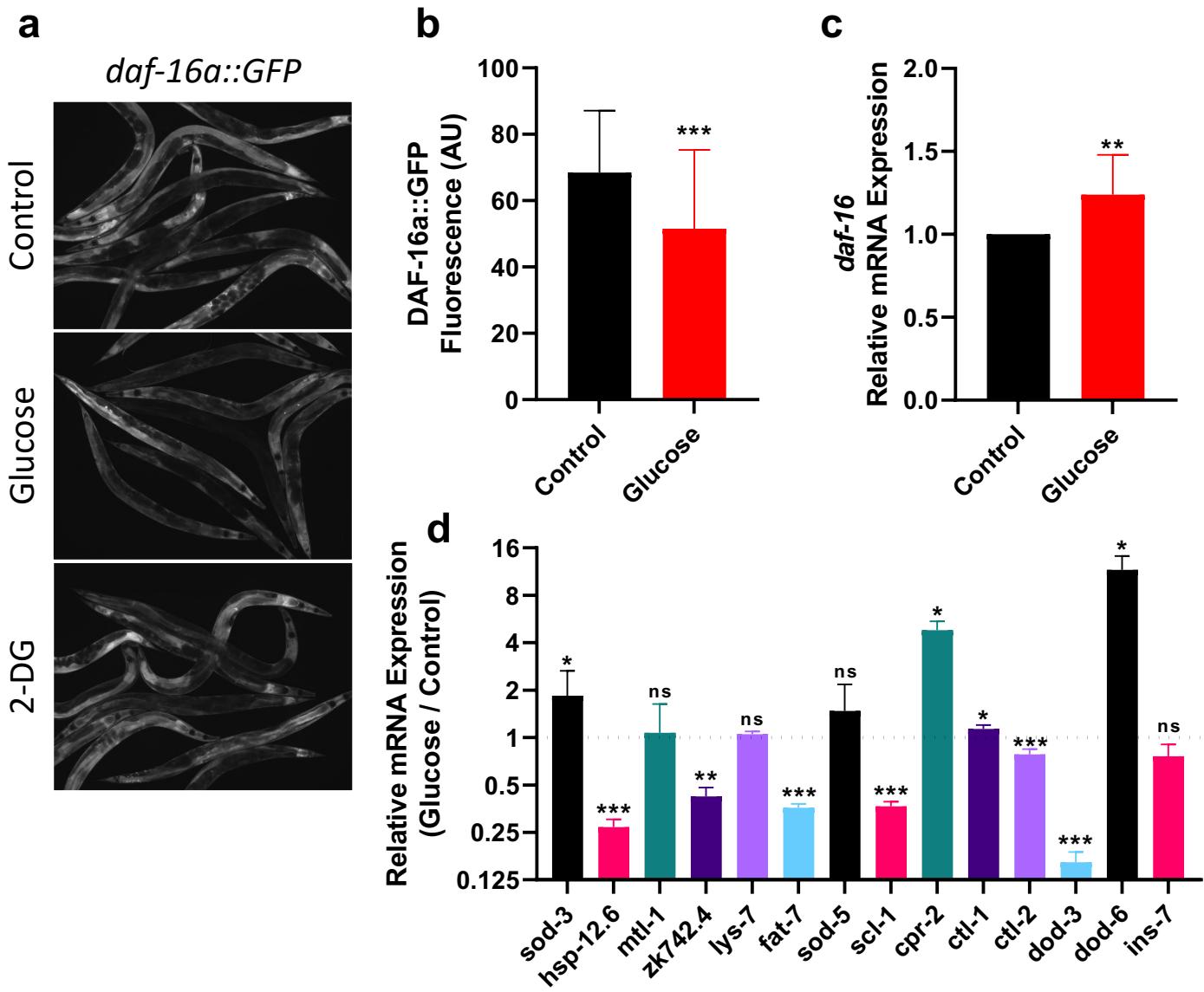
Supplementary Figure S1. Effects of Adding Glucose Directly to *E. coli*. (a, b—*E. coli*, c—*C. elegans*) (a) Optical Density (600nm) of 0% control and 0.4% glucose fed OP50 *E. coli* over 24 hr ($P \leq 0.001$ ***), 48 hr ($P \leq 0.05$ *) and 72 hr ($P > 0.05$ ns). (b) Glucose assay of 0% control and 0.4% glucose fed OP50 *E. coli* after 3 days in LB, normalized to protein concentration. (c) *C. elegans* body size over time consuming 0% control and 0.4% glucose fed OP50 *E. coli* measured from photos and quantified by pixel area per animal, Day 1 *C. elegans* consuming 0% control ($n=27$) and 0.4% glucose fed OP50 *E. coli* ($n=30$), ** $P \leq 0.005$; Day 6 *C. elegans* consuming 0% control ($n=177$) and 0.4% glucose fed OP50 *E. coli* ($n=186$), *** $P \leq 0.001$; Day 12 *C. elegans* consuming 0% control ($n=62$) and 0.4% glucose fed OP50 *E. coli* ($n=66$), *** $P \leq 0.001$. All Statistical analysis of *C. elegans* consuming either 0% control or 0.4% glucose fed OP50 *E. coli* compared at the same time point using an unpaired two-tailed t test with Graphpad Prism 8 software (ns = not significant $P > 0.05$, * $P \leq 0.05$, ** $P \leq 0.005$, *** $P \leq 0.001$). Data shown is a compilation from at least 3 biological replicates.



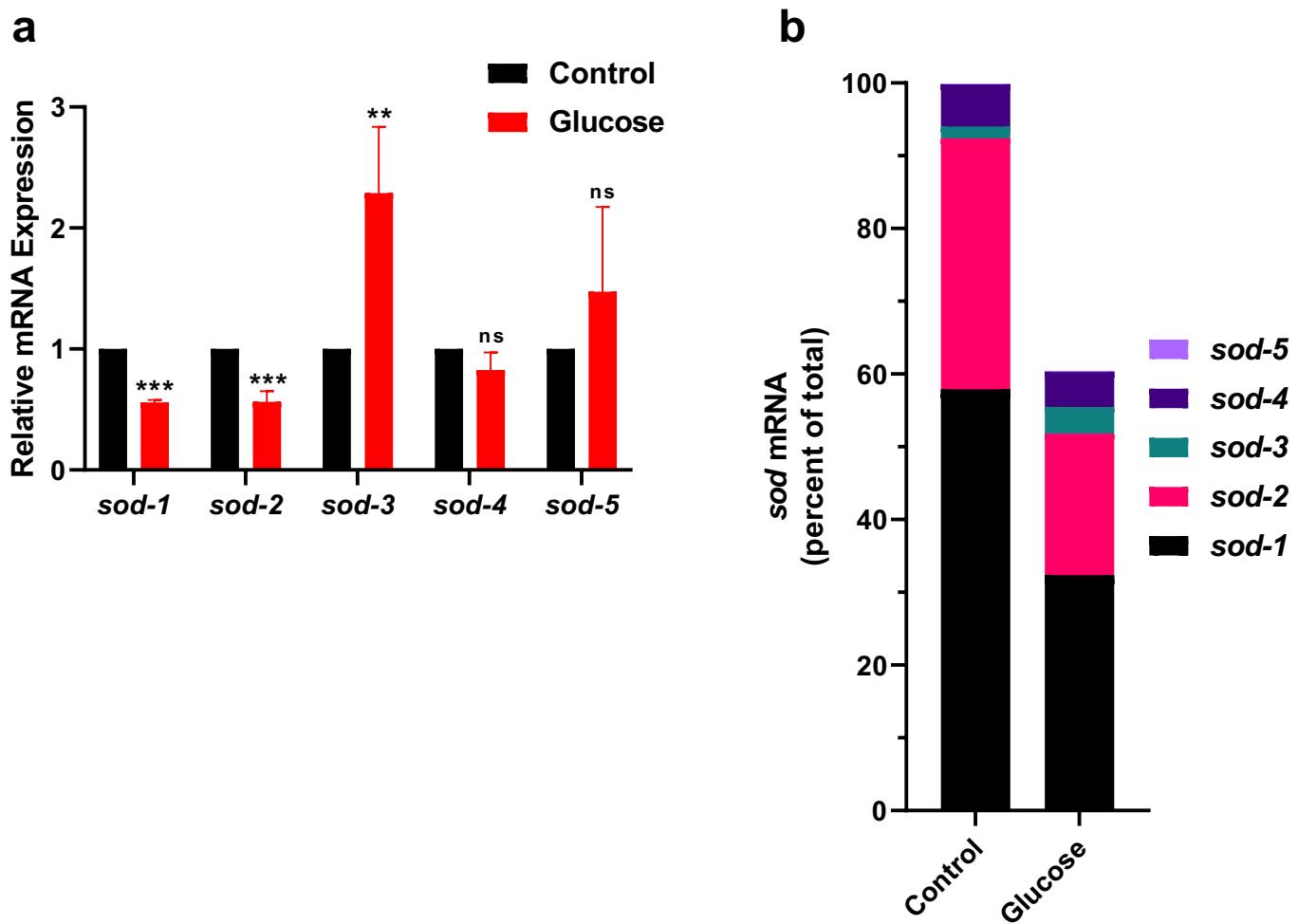
Supplementary Figure S2. Interfering with bacterial metabolism of glucose alters *C. elegans* physiology. (a) Healthspan – Movement in liquid/thrashing/swimming of wild type *C. elegans* consuming either 0% control ($n=53$), 0.4% glucose pre culture ($n=54$) or 0.4% glucose post culture ($n=54$) supplemented OP50 *E. coli* over time. (b) Healthspan – Movement in liquid/thrashing/swimming of wild type *C. elegans* consuming either 0% control ($n=30$), 0.4% glucose ($n=32$), or 0.4% 2-deoxy-glucose (2-DG; $n=31$) fed OP50 *E. coli* over time. (c) Lifespan of wild type *C. elegans* with 0, 0.6, 6, and 30mM Carnosine within the agar seeded with heat killed OP50 *E. coli*. The mean lifespans were as follows: Mean \pm Standard Deviation ($n=$ total number of animals assayed); wild type *C. elegans* with 0 mM Carnosine 18.7 ± 0.63 ($n=60$); wild type *C. elegans* with 0.6 mM Carnosine 19.4 ± 0.73^{ns} ($n=54$); wild type *C. elegans* with 6mM Carnosine 18.6 ± 0.56^{ns} ($n=53$); wild type *C. elegans* with 30mM Carnosine $10.1 \pm 1.2^{***}$ ($n=60$). All statistical analysis of histograms compares *C. elegans* consuming either 0% control or 0.4% glucose fed OP50 *E. coli* at the same time point using an unpaired two-tailed t test, and survival graph performed using a Log-rank Mantel-Cox test with Graphpad Prism 8 software (ns = not significant, * $P \leq 0.05$, ** $P \leq 0.005$, *** $P \leq 0.001$). Data shown is a compilation from at least 3 biological replicates.

a*skn-1::GFP**gcs-1::GFP**hsp-16.2::GFP***b**

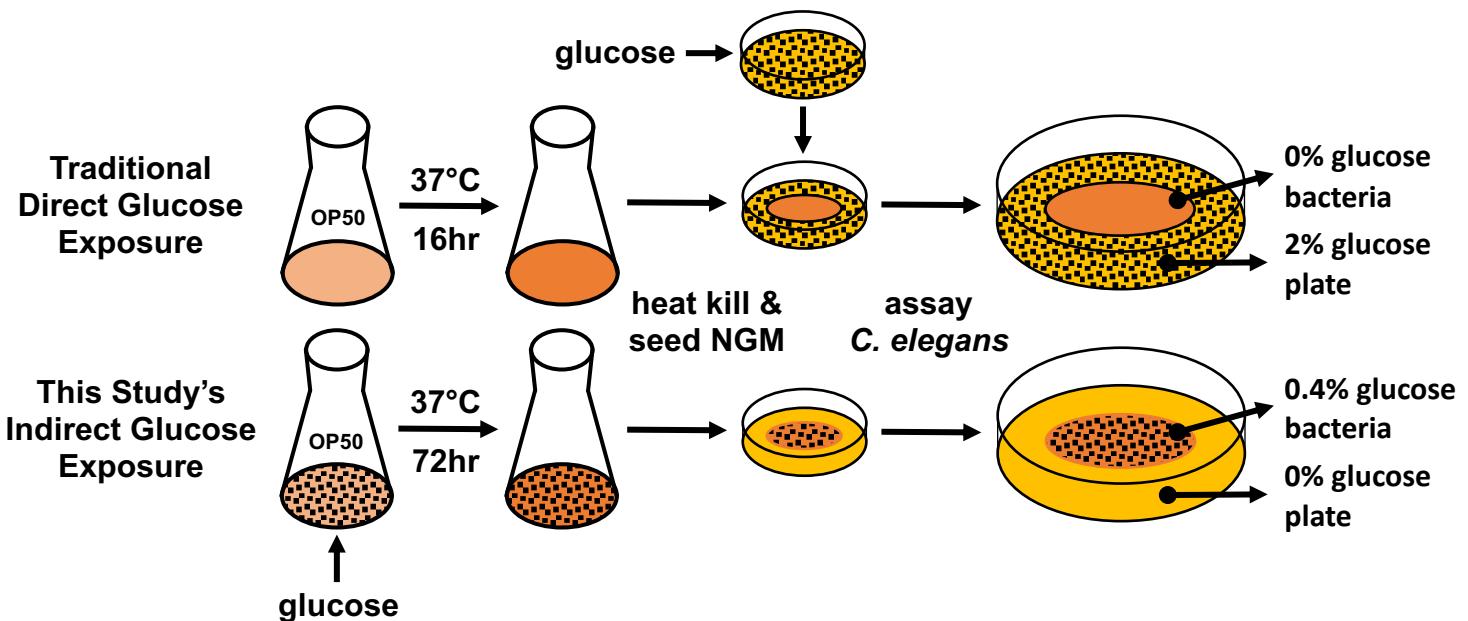
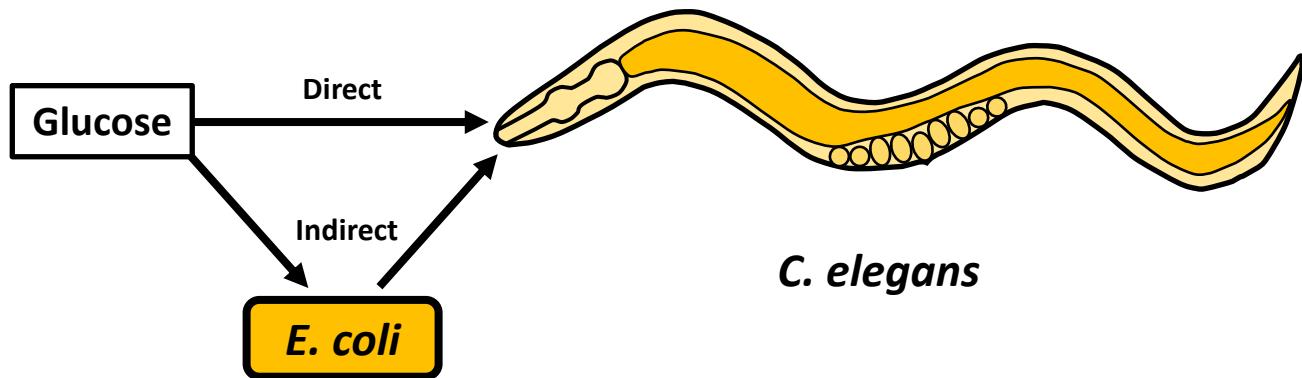
Supplementary Figure S3. *C. elegans* gene expression changes with bacterial metabolism of glucose. (a) Fluorescent imaging of transgenic *C. elegans* [*skn-1::gfp*], [*gcs-1::gfp*], or [*hsp-16.2::gfp*], after consuming either 0% control, 0.4% glucose, or 0.4% 2-deoxy-glucose (2-DG) fed OP50 *E. coli* for 6 days. (b) RTqPCR of wild type *C. elegans* consuming either 0% control or 0.4% glucose fed *E. coli* for 6 days displayed as relative expression of glucose fed *E. coli* compared to control. Statistical analysis performed using an unpaired two-tailed t test with Graphpad Prism 8 software (ns = not significant, *P ≤ 0.05, **P ≤ 0.005, ***P ≤ 0.001). All of the data was compiled from at least 3 biological replicates.



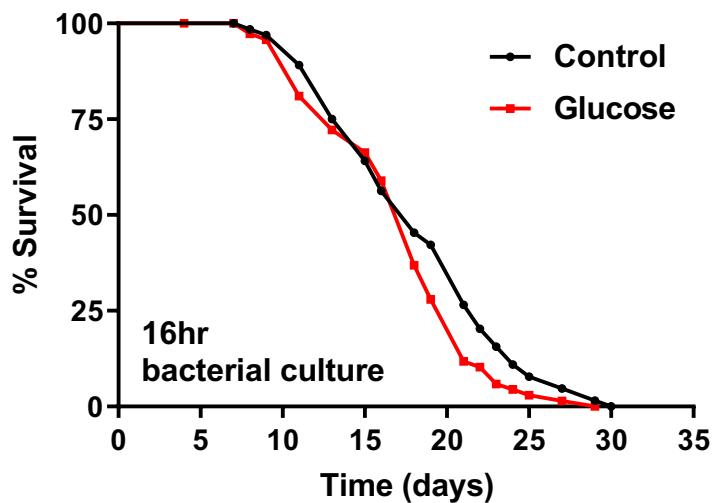
Supplementary Figure S4. *C. elegans* *daf-16* expression with bacterial metabolism of glucose. (a) Fluorescent imaging of transgenic *C. elegans* [*daf-16a::gfp*], after consuming either 0% control, 0.4% glucose, or 0.4% 2-deoxy-glucose (2-DG) fed OP50 *E. coli* for 6 days. (b) Fluorescence quantification of *daf-16a::gfp* animals in Figure S4a after consuming 0% control (n=40) and 0.4% glucose (n=40) fed OP50 *E. coli* for 6 days. (c) RTqPCR of *daf-16* mRNA in wild type *C. elegans* consuming either 0% control or 0.4% glucose fed *E. coli* for 6 days. (d) RTqPCR of *daf-16* target genes in wild type *C. elegans* consuming either 0% control or 0.4% glucose fed *E. coli* for 6 days, displayed as relative expression of glucose treatment compared to control. Statistical analysis performed using an unpaired two-tailed t test with Graphpad Prism 8 software (ns = not significant, *P ≤ 0.05, **P ≤ 0.005, ***P ≤ 0.001). Data shown was collected from at least 2 technical repeats.



Supplementary Figure S5. *C. elegans* sod mRNA expression with bacterial metabolism of glucose. (a) RTqPCR experiments performed using wild type *C. elegans* consuming either 0% control and 0.4% glucose fed *E. coli* for 6 days, examining the 5 sod genes. (b) Estimated percent of total sod expression within *C. elegans* treated with glucose fed *E. coli* based off the preceding RTqPCR data and published RNA Seq expression ratio data (Dues et al 2017). Statistical analysis performed using an unpaired two-tailed t test with Graphpad Prism 8 software (ns = not significant, *P ≤ 0.05, **P ≤ 0.005, ***P ≤ 0.001), all experimental data collected from at least 3 biological replicates.



Supplementary Figure S6. Model illustration of direct and indirect glucose supplementation to *C. elegans*, and a diagram of the protocols that achieve either type of exposure method.



Supplementary Figure S7. Overnight (16hr) incubation of *E. coli* with glucose does not affect *C. elegans* lifespan. Lifespan of wild type *C. elegans* consuming either 0% control or 0.4% glucose fed OP50 *E. coli* for 16 hours. The mean lifespans were as follows: Mean \pm Standard Deviation (n= total number of animals assayed) wild type *C. elegans* treated with 16 hour cultured control 18.3 ± 0.7 (n=67) and 0.4% glucose 17.1 ± 0.6^{ns} (n=77) fed OP50 *E. coli*. Statistical analysis performed using a Log-rank Mantel-Cox test with Graphpad Prism 8 software (ns = not significant). Data is a compilation of at least 3 biological replicates.

Supplementary Table S1: Summary of pooled Lifespan statistics of *C. elegans* on all treatments

P values calculated against the appropriate control treatment using a Log-rank Mantel-Cox test.
(*P ≤ 0.05, **P ≤ 0.005, ***P ≤ 0.001, ns = not significant)

Figure	<i>C. elegans</i> Genotype	<i>E. coli</i> Treatment	Mean Survival ± Std Dev (Days)	P value	P *	Animal Deaths	Censored Subjects	Repeats
1D	Wild type (N2)	0% Control	21.2 ± 2.1	-		955	50	12
		0.4% Glucose	17.1 ± 2.9	0.0001	***	879	72	
2A	Wild type (N2)	0% Control	21.4 ± 1.6	-		361	14	4
		0.4% Glucose	17.9 ± 2.6	0.0001	***	345	9	
		0.4% Glucose Post Culture	20.6 ± 1.2	0.0199	*	354	17	
2B	Wild type (N2)	0% Control	21.4 ± 0.2	-		275	15	3
		0.4% Glucose	16.6 ± 2.3	0.0001	***	165	8	
		0.4% 2-DG	21.6 ± 1.2	0.0134	*	288	12	
2C	Wild type (N2)	0% Control	20.9 ± 1.9	-		238	16	3
		0.4% Glucose	15.4 ± 1.8	0.0001	***	207	28	
		50mM Carnosine	22.3 ± 1.3	0.0001	***	208	23	
		50mM Carnosine + 0.4% Glucose	19.4 ± 1.8	0.0001	***	244	26	
4B	Wild type (N2)	0% Control	19.1 ± 1.6	-		257	9	3
	<i>gst-4(lp11)</i>	0% Control	17.2 ± 1.2	0.0001	***	251	10	
	<i>gst-4(lp10)</i>	0% Control	17.3 ± 0.0	0.0001	***	90	2	
4C	Wild type (N2)	0% Control	20.3 ± 1.0	-		290	15	3
		0.4% Glucose	18.4 ± 0.5	0.0001	***	128	16	
	<i>gst-4(lp11)</i>	0% Control	19.0 ± 0.6	-		298	12	
		0.4% Glucose	18.9 ± 1.1	0.7508	ns	302	9	

Supplementary Table S2: Individual Lifespan statistics of *C. elegans* on all treatments.

* P values calculated against the appropriate control using a Log-rank Mantel-Cox test

(*P ≤ 0.05, **P ≤ 0.005, ***P ≤ 0.001, ns = not significant)

Figure	<i>C. elegans</i> Genotype	<i>E. coli</i> Treatment	Mean Survival (Days)	P value	P *	Animal Deaths	Censored Subjects	Repeat #
1D	Wild type (N2)	0% Control	22.3	-		81	5	1
		0.4% Glucose	19.2	0.0031	**	68	1	
1D	Wild type (N2)	0% Control	20.0	-		60	2	2
		0.4% Glucose	18.3	0.0335	*	59	2	
1D	Wild type (N2)	0% Control	19.6	-		107	2	3
		0.4% Glucose	13.7	0.0001	***	102	2	
1D	Wild type (N2)	0% Control	23.5	-		113	5	4
		0.4% Glucose	20.7	0.0001	***	116	4	
1D	Wild type (N2)	0% Control	18.2	-		61	2	5
		0.4% Glucose	15.8	0.0736	ns	32	2	
1D	Wild type (N2)	0% Control	19.5	-		91	2	6
		0.4% Glucose	15.0	0.0001	***	109	3	
1D	Wild type (N2)	0% Control	23.4	-		70	1	7
		0.4% Glucose	15.2	0.0001	***	52	15	
1D	Wild type (N2)	0% Control	20.3	-		60	12	8
		0.4% Glucose	17.7	0.2699	ns	57	10	
1D	Wild type (N2)	0% Control	25.5	-		42	4	9
		0.4% Glucose	23.0	0.6152	ns	21	22	
1D	Wild type (N2)	0% Control	19.0	-		108	3	10
		0.4% Glucose	13.2	0.0001	***	98	3	

Supplementary Table S2: Individual Lifespan statistics of *C. elegans* on all treatments.

* P values calculated against the appropriate control using a Log-rank Mantel-Cox test.

(*P ≤ 0.05, **P ≤ 0.005, ***P ≤ 0.001, ns = not significant)

Figure	<i>C. elegans</i> Genotype	<i>E. coli</i> Treatment	Mean Survival (Days)	P value	P *	Animal Deaths	Censored Subjects	Repeat #
1D	Wild type (N2)	0% Control	21.6	-		83	9	11
		0.4% Glucose	18.9	0.0001	***	89	5	
1D	Wild type (N2)	0% Control	21.4	-		79	3	12
		0.4% Glucose	14.4	0.0001	***	76	3	
2A	Wild type (N2)	0% Control	22.3	-		81	5	1
		0.4% Glucose	19.2	0.0031	**	68	1	
		0.4% Glucose Post Culture	21.2	0.254	ns	83	10	
2A	Wild type (N2)	0% Control	20.0	-		60	2	2
		0.4% Glucose	18.3	0.0335	*	59	2	
		0.4% Glucose Post Culture	19.5	0.3266	ns	62	2	
2A	Wild type (N2)	0% Control	19.6	-		107	2	3
		0.4% Glucose	13.7	0.0001	***	102	2	
		0.4% Glucose Post Culture	19.4	0.9425	ns	106	2	
2A	Wild type (N2)	0% Control	23.5	-		113	5	4
		0.4% Glucose	20.7	0.0001	***	116	4	
		0.4% Glucose Post Culture	22.3	0.0338	*	103	3	
2B	Wild type (N2)	0% Control	21.6	-		83	9	1
		0.4% Glucose	18.9	0.0001	***	89	5	
		0.4% 2-DG	19.9	0.0015	**	83	6	

Supplementary Table S2: Individual Lifespan statistics of *C. elegans* on all treatments.

* P values calculated against the appropriate control using a Log-rank Mantel-Cox test.

(*P ≤ 0.05, **P ≤ 0.005, ***P ≤ 0.001, ns = not significant)

Figure	<i>C. elegans</i> Genotype	<i>E. coli</i> Treatment	Mean Survival (Days)	P value	P *	Animal Deaths	Censored Subjects	Repeat #
2B	Wild type (N2)	0% Control	21.4	-		79	3	2
		0.4% Glucose	14.4	0.0001	***	76	3	
		0.4% 2-DG	22.4	0.0175	*	94	3	
2B	Wild type (N2)	0% Control	21.1	-		113	3	3
		0.4% Glucose				0	0	
		0.4% 2-DG	22.3	0.0041	**	110	3	
2C	Wild type (N2)	0% Control	23.4	-		70	1	1
		0.4% Glucose	15.2	0.0001	***	52	15	
		50mM Carnosine	22.0	0.1111	ns	65	3	
		50mM Carnosine + 0.4% Glucose	19.1	0.0001	***	69	1	
2C	Wild type (N2)	0% Control	19.0	-		108	3	2
		0.4% Glucose	13.2	0.0001	***	98	3	
		50mM Carnosine	20.9	0.0032	**	87	3	
		50mM Carnosine + 0.4% Glucose	17.4	0.002	**	125	3	
2C	Wild type (N2)	0% Control	20.3	-		60	12	3
		0.4% Glucose	17.7	0.2699	ns	57	10	
		50mM Carnosine	24.0	0.0001	***	56	17	
		50mM Carnosine + 0.4% Glucose	21.8	0.0256	*	49	22	
4B	Wild type (N2)	0% Control	16.9	-		67	3	1
	gst-4(lp11)	0% Control	16.1	0.0557	ns	61	5	
	gst-4(lp10)	0% Control				0	0	

Supplementary Table S2: Individual Lifespan statistics of *C. elegans* on all treatments.

* P values calculated against the appropriate control using a Log-rank Mantel-Cox test.

(*P ≤ 0.05, **P ≤ 0.005, ***P ≤ 0.001, ns = not significant)

Figure	<i>C. elegans</i> Genotype	<i>E. coli</i> Treatment	Mean Survival (Days)	P value	P *	Animal Deaths	Censored Subjects	Repeat #
4B	Wild type (N2)	0% Control	20.0	-		86	3	2
	<i>gst-4(lp11)</i>	0% Control	16.8	0.0001	***	86	3	
	<i>gst-4(lp10)</i>	0% Control	17.3	0.0001	***	90	2	
4B	Wild type (N2)	0% Control	20.3	-		104	3	3
	<i>gst-4(lp11)</i>	0% Control	18.8	0.0011	**	104	3	
	<i>gst-4(lp10)</i>	0% Control				0	0	
4C	Wild type (N2)	0% Control	21.6	-		83	9	1
		0.4% Glucose	18.9	0.0001	***	89	5	
	<i>gst-4(lp11)</i>	0% Control	19.8	-		85	6	
		0.4% Glucose	20.2	0.0894	ns	88	3	
4C	Wild type (N2)	0% Control	19.1	-		103	3	2
		0.4% Glucose				0	0	
	<i>gst-4(lp11)</i>	0% Control	18.4	-		109	3	
		0.4% Glucose	17.6	0.0638	ns	112	3	
4C	Wild type (N2)	0% Control	20.3	-		104	3	3
		0.4% Glucose	17.9	0.0001	***	39	11	
	<i>gst-4(lp11)</i>	0% Control	18.8	-		104	3	
		0.4% Glucose	18.9	0.7574	ns	102	3	

Supplementary Table S3: RT-qPCR, PCR, and CRISPR primer sequences

Gene	Use	Sequence	Source
<i>act-1</i>	RT-qPCR	CTCTTGCCCCATCAACCATG	Kwon et al 2010
<i>act-1</i>	RT-qPCR	CTTGCTTGGAGATCCACATC	Kwon et al 2010
<i>act-1</i>	RT-qPCR	GGAGTCATGGTCGGTATGG	GETprime Ensembl release 81
<i>act-1</i>	RT-qPCR	CTTGAGGGTAAGGATAACCTCTC	GETprime Ensembl release 81
<i>atfs-1</i>	RT-qPCR	TTGGAGATAATATGGGCTCCC	GETprime Ensembl release 81
<i>atfs-1</i>	RT-qPCR	CTATTGGGAAGTTCCCGT	GETprime Ensembl release 81
<i>cpr-2</i>	RT-qPCR	CTGCGTAAACCTTCAAACTC	Kwon et al 2010
<i>cpr-2</i>	RT-qPCR	ATGCGGAGTTACCATAGTTC	Kwon et al 2010
<i>ctl-1</i>	RT-qPCR	CCAATGCTCATGCAAGATGT	Schaar et al 2015
<i>ctl-1</i>	RT-qPCR	TTGCGTCACGAATGAAGAAG	Schaar et al 2015
<i>ctl-2</i>	RT-qPCR	ACGTCCCTGGAGCATCTTGT	Schaar et al 2015
<i>ctl-2</i>	RT-qPCR	GCAAGATGGTGCTAACAGA	Schaar et al 2015
<i>ctl-3</i>	RT-qPCR	CTTCCCCACATGGTCAATCT	Schaar et al 2015
<i>ctl-3</i>	RT-qPCR	TGTCCTGCATTAGCATTGGA	Schaar et al 2015
<i>daf-16</i>	RT-qPCR	TCCATCATCTTCCGTCCC	GETprime Ensembl release 81
<i>daf-16</i>	RT-qPCR	CTTCCAATAGCTGGAGAACAC	GETprime Ensembl release 81
<i>djr-1.1</i>	RT-qPCR	TTGAGCCATGGAGTCAAGG	GETprime Ensembl release 81
<i>djr-1.1</i>	RT-qPCR	AGTACTTGTAGCCTCCTTCTC	GETprime Ensembl release 81
<i>djr-1.2</i>	RT-qPCR	CTGAACCTGTCAAATGTGCC	GETprime Ensembl release 81
<i>djr-1.2</i>	RT-qPCR	TGTCGAATGTCTTGTCTTCAC	GETprime Ensembl release 81
<i>dod-3</i>	RT-qPCR	GTGCATATTGTGGAGCTGC	GETprime Ensembl release 81
<i>dod-3</i>	RT-qPCR	ATAGTTGGTCGGACGTTGG	GETprime Ensembl release 81
<i>dod-6</i>	RT-qPCR	GATGCTGAAGATGGTCACTG	GETprime Ensembl release 81
<i>dod-6</i>	RT-qPCR	AAGTCTTGGGCACTTGGA	GETprime Ensembl release 81
<i>fat-7</i>	RT-qPCR	ATAGTGTGGCGTAACGTGGC	Kwon et al 2010
<i>fat-7</i>	RT-qPCR	TAGAGAGCAAATGAGAACG	Kwon et al 2010
<i>gcs-1</i>	RT-qPCR	CCAATCGATTCCCTTGGAGAC	GETprime Ensembl release 81
<i>gcs-1</i>	RT-qPCR	CGATGAGACCTCCGTAAGG	GETprime Ensembl release 81
<i>gss-1</i>	RT-qPCR	AAGGAAGGATGCACCTGAG	GETprime Ensembl release 81
<i>gss-1</i>	RT-qPCR	GCTACTCCACCCATAGCTG	GETprime Ensembl release 81
<i>gst-10</i>	RT-qPCR	CTTCACTATTGAGGATTGG	GETprime Ensembl release 81
<i>gst-10</i>	RT-qPCR	TCGAACCGAATGTCTTCGA	GETprime Ensembl release 81
<i>gst-29</i>	RT-qPCR	ATGGAGATGGTACATGGGA	GETprime Ensembl release 81
<i>gst-29</i>	RT-qPCR	TAGAACTGGAACCTGGCCA	GETprime Ensembl release 81
<i>gst-4</i>	RT-qPCR	GCTGAAGCCAACGACTCCAT	Park, S-K. et al 2009
<i>gst-4</i>	RT-qPCR	GACCGAATTGTTCTCCATCGA	Park, S-K. et al 2009
<i>gst-4</i>	CRISPR	AAATACAATAGCTTATAGTT	http://crispor.tefor.net
<i>gst-4</i>	CRISPR	TCGTTGGAGACTCATTGACT	http://crispor.tefor.net
<i>gst-4</i>	PCR	TGATGCCAGACGATGACATTAC	IDT dna PrimerQuest tool
<i>gst-4</i>	PCR	TCTCTGGGAGACGTGATAGG	IDT dna PrimerQuest tool
<i>gst-4</i>	PCR	CAAATTCCAGCGACTCCATT	IDT dna PrimerQuest tool
<i>gst-4</i>	PCR	ATGATCAGCGTCACTCCATAG	IDT dna PrimerQuest tool

Supplementary Table S3: RT-qPCR, PCR, and CRISPR primer sequences

Gene	Use	Sequence	Source
<i>hsf-1</i>	RT-qPCR	TCAGACAGTTGAATATGTACGG	GETprime Ensembl release 81
<i>hsf-1</i>	RT-qPCR	CCTGATCTGATTCTGTCGAG	GETprime Ensembl release 81
<i>hsp-12.6</i>	RT-qPCR	TGGAGTTGTCAATGTCCTCG	Kwon et al 2010
<i>hsp-12.6</i>	RT-qPCR	GACTTCAATCTCTTGGGAGG	Kwon et al 2010
<i>hsp-16.2</i>	RT-qPCR	TGGTGCAGTTGCTTCGAATC	Park, S-K. et al 2009
<i>hsp-16.2</i>	RT-qPCR	TTGAACCGCTTCTTCTTGG	Park, S-K. et al 2009
<i>hsp-6</i>	RT-qPCR	GAAGATAACGAAGACCCAGAGGTT	Pellegrino MW et al 2014
<i>hsp-6</i>	RT-qPCR	CAACCTGAGATGGGAATACACT	Pellegrino MW et al 2014
<i>ins-7</i>	RT-qPCR	AGAACCGAGAAGAGTCCTGA	Kwon et al 2010
<i>ins-7</i>	RT-qPCR	ATATTAACCTCAGTATTGATTG	Kwon et al 2010
<i>lys-7</i>	RT-qPCR	GCCGTCAAACCTGGCATCTT	Kwon et al 2010
<i>lys-7</i>	RT-qPCR	GGGTTGTATGCACGAACGAA	Kwon et al 2010
<i>mtl-1</i>	RT-qPCR	AATCATGGCTTGCAAGTGTG	GETprime Ensembl release 81
<i>mtl-1</i>	RT-qPCR	TTCACATTGTCCTCCGCAC	GETprime Ensembl release 81
<i>oga-1</i>	RT-qPCR	ACATTATGTGGACAGGACCTC	GETprime Ensembl release 81
<i>oga-1</i>	RT-qPCR	GACGCATTACACTTCCCAC	GETprime Ensembl release 81
<i>ogt-1</i>	RT-qPCR	TGATCATGACAGGACAAATGAC	GETprime Ensembl release 81
<i>ogt-1</i>	RT-qPCR	GATGCATTGAGACTGTCCG	GETprime Ensembl release 81
<i>ostb-1</i>	RT-qPCR	ATGATGTGCAACAGGTGTC	GETprime Ensembl release 81
<i>ostb-1</i>	RT-qPCR	CGTAGTATGGATATGCGGAG	GETprime Ensembl release 81
<i>pept-1</i>	RT-qPCR	TGCAACACTGGTATTATGGG	GETprime Ensembl release 81
<i>pept-1</i>	RT-qPCR	CGAGATACTCTCCGAACAC	GETprime Ensembl release 81
<i>prdx-2</i>	RT-qPCR	GGCCTGAACAAGACGAAGAG	Schaar et al 2015
<i>prdx-2</i>	RT-qPCR	GTGTGCCAACCGAGATTAT	Schaar et al 2015
<i>prdx-3</i>	RT-qPCR	CAGCTCCAAGAGAACGGAAC	Schaar et al 2015
<i>prdx-3</i>	RT-qPCR	CCAGCTTCAAAGGAACTGC	Schaar et al 2015
<i>prdx-6</i>	RT-qPCR	TGCTGTGATGCTTTGGAC	Schaar et al 2015
<i>prdx-6</i>	RT-qPCR	TCAGAGATGGTTGAGCGATG	Schaar et al 2015
<i>scl-1</i>	RT-qPCR	CAATCAAGCATTGTGGATGC	Kwon et al 2010
<i>scl-1</i>	RT-qPCR	GGAATCCACGACCATTTC	Kwon et al 2010
<i>skn-1</i>	RT-qPCR	GCAACAGCTACTCAATCGT	GETprime Ensembl release 81
<i>skn-1</i>	RT-qPCR	TGATGACGAATCAGTAGTGC	GETprime Ensembl release 81
<i>sod-1</i>	RT-qPCR	ACTGTCGTTGGCCGATCTATG	Schaar et al 2015
<i>sod-1</i>	RT-qPCR	CGCCGAGGTCGTCTTGT	Schaar et al 2015
<i>sod-2</i>	RT-qPCR	CAAAGGACGGAGGAGAACCA	Schaar et al 2015
<i>sod-2</i>	RT-qPCR	CGCTCTTAATTGCGGTGAGC	Schaar et al 2015
<i>sod-3</i>	RT-qPCR	AAAGGAGCTGATGGACACTATTAAGC	Schaar et al 2015
<i>sod-3</i>	RT-qPCR	AAGTTATCCAGGGAACCGAAGTC	Schaar et al 2015
<i>sod-4</i>	RT-qPCR	TTGAAGCCGCTTCCGAAG	Schaar et al 2015
<i>sod-4</i>	RT-qPCR	CAGCTTGAAAATGTAGGCACGT	Schaar et al 2015
<i>sod-5</i>	RT-qPCR	CTGTTATCGGACGTTCCATGGT	Schaar et al 2015
<i>sod-5</i>	RT-qPCR	GGCCAAGATCATCTCGATCG	Schaar et al 2015

Supplementary Table S3: RT-qPCR, PCR, and CRISPR primer sequences			
Gene	Use	Sequence	Source
<i>trx-1</i>	RT-qPCR	CGTCAACATCCGGAGAAGAT	Schaar et al 2015
<i>trx-1</i>	RT-qPCR	TGCTCCAACACTTTTGACG	Schaar et al 2015
<i>trx-2</i>	RT-qPCR	GTTGATTCCACGCAGAATG	Schaar et al 2015
<i>trx-2</i>	RT-qPCR	TGGCGAGAAGAACACTTCCT	Schaar et al 2015
<i>trx-3</i>	RT-qPCR	TGGTAGATGCAGGAGAGCAC	Schaar et al 2015
<i>trx-3</i>	RT-qPCR	TCCAAGTGAATCGCATGTA	Schaar et al 2015
<i>ugt-41</i>	RT-qPCR	CTCTTCTAGCTGATTCCGT	GETprime Ensembl release 81
<i>ugt-41</i>	RT-qPCR	TTCCGAGGTAGCTAACTC	GETprime Ensembl release 81
ZK742.4	RT-qPCR	ACTGGAGGTTGGCAAAGTGC	Kwon et al 2010
ZK742.4	RT-qPCR	TACGAGGCAAATCTGGCTCA	Kwon et al 2010

References

1. Kwon, E. S., Narasimhan, S. D., Yen, K. & Tissenbaum, H. A. A new DAF-16 isoform regulates longevity. *Nature* **466**, 498-502, doi:10.1038/nature09184 (2010).
2. Schaar, C. E. et al. Mitochondrial and cytoplasmic ROS have opposing effects on lifespan. *PLoS Genet* **11**, e1004972, doi:10.1371/journal.pgen.1004972 (2015).
3. Park, S. K., Tedesco, P. M. & Johnson, T. E. Oxidative stress and longevity in *Caenorhabditis elegans* as mediated by SKN-1. *Aging Cell* **8**, 258-269, doi:ACE473 [pii]10.1111/j.1474-9726.2009.00473.x (2009).
4. Pellegrino, M. W. et al. Mitochondrial UPR-regulated innate immunity provides resistance to pathogen infection. *Nature* **516**, 414-417, doi:10.1038/nature13818 (2014).